RECEIVED **CENTRAL FAX CENTER** SEP 0 6 2006

Amendment to the Claims

650-845-6504

Claims 1 - 4. (Canceled)

- 5. (Currently amended): An isolated nucleic acid molecule which encodes a polypeptide having an amino acid sequence of SEQ ID NO: 12 or an amino acid sequence having at least 40% 95% sequence identity thereto, wherein said polypeptide is a transmembrane protein which has 2,5-diketo-gluconate 2,5-diketo-Dgluconic acid (2,5-DKG) permease activity.
- 6. (Canceled)
- 7. (Previously presented): The isolated nucleic acid molecule of claim 5, which encodes a polypeptide having the amino acid sequence of SEQ ID NO: 12.

Claim 8 - 10. (Canceled)

- 11. (Currently amended): An isolated nucleic acid molecule comprising a polynucleotide which encodes a polypeptide having an amino acid sequence of SEQ ID NO. 12 or an amino acid sequence having at least 40% 95% sequence identity thereto, wherein said polypeptide has 2,5-diketo-gluconate 2,5-diketo-D-gluconic acid (2,5-DKG) permease activity, and wherein the polynucleotide is operatively linked to a promoter of gene expression.
- The isolated nucleic acid molecule of claim 11, wherein 12. (Original): said promoter is a lac promoter.
- A vector comprising the isolated nucleic acid molecule of 13. (Original): claim 11.

14. (Original):

The vector of claim 13, comprising a spectromycin

resistance gene.

15. (Original):

A bacterial cell, comprising the vector of claim 13.

Claims 16 - 19. (Canceled)

20. (Original): The bacterial cell of claim 15, which is of the genus Klebsiella.

21. (Currently amended): The bacteria cell of claim 15, which is deficient in endogenous 2,5-DKG <u>permease</u> activity.

- 22. (Currently amended): The bacterial cell of claim 21, further comprising an isolated nucleic acid molecule encoding a polypeptide having 2-keto reductase activity, said polypetide having at least 95% and at least 80% sequence identity to SEQ ID NO: 14.
- 23. (Currently amended): The bacterial cell of claim 21, further comprising an isolated nucleic acid molecule encoding a polypeptide having 5-keto reductase activity, said polypetide having at least 95% and at least 80% sequence identity to SEQ ID NO: 16.

24. (Original):

The bacterial cell of claim 15, which is of the genus

Pantoea.

25. (Previously presented): The bacterial cell of claim 15, which expresses an enzyme that catalyzes the conversion of 2,5-DKG to 2-keto-L-gulonic acid (2-KLG).

26. (Original): The bacterial cell of claim 25, which expresses enzymes that catalyze the conversion of glucose to 2,5-DKG.

27. (Original): The bacterial cell of claim 26, which is deficient in endogenous 2-keto-reductase activity.

PAGE 07/16

Claims 28 – 35. (Canceled)

- 36. (Currently amended): A method of <u>enhancing</u>
 <u>using the isolated nucleic acid molecule of claim 5 to enhance</u> 2-keto-L-gulonic acid
 (2-KLG) production, comprising
- a) introducing the isolated an isolated nucleic acid molecule of claim-5 encoding a polypeptide having at least 95% sequence identity to SEQ ID NO: 12 into a bacterial cell which expresses an enzyme that catalyzes the conversion of 2,5-diketo-D-gluconic acid (2,5-DKG) to 2-KLG, b) allowing expression of the polypeptide encoded by said nucleic acid molecule and c) culturing the bacterial cell under suitable conditions to produce 2-KLG.
- 37. (Original): The method of claim 36, wherein said bacterial cell further expresses enzymes that catalyze the conversion of glucose to 2,5-DKG.
- 38. (Original): The method of claim 37, wherein said bacterial cell is deficient in endogenous 2-keto reductase activity.
- 39. (Original): The method of claim 36, wherein said bacterial cell is of the genus *Pantoea*.
- 40. (Original): The method of claim 36, further comprising converting said 2-KLG to ascorbic acid.

Claims 41 – 48. (Canceled)

- 49, (Previously presented):
- The bacterial cell of claim 15, which is an E. coli cell.

- 50. (Canceled)
- 51. (Previously presented): The method of claim 36, wherein the nucleic acid molecule has the sequence of SEQ ID NO: 11 or a sequence having at least 95% sequence identity thereto.
- 52. (Currently amended): A method for increasing the transport of 2, 5-DKG 2, 5 diketo-D-gluconic acid (2, 5 DKG) across a cell membrane into a bacterial host cell comprising a) introducing the an isolated nucleic acid molecule of claim 5 having 2,5-DKG permease activity into a bacterial host cell, wherein the nucleic acid molecule encodes a protein comprising at least 95% sequence identity to SEQ ID NO: 12 and said protein having 2,5 DKG permease activity, b) allowing expression of the 2,5-DKG permease protein and c) culturing the bacterial host cell under suitable conditions for the transport of 2,5-DKG into the bacterial host cell.
- 53. (Previously presented): The method according to claim 52, wherein the bacterial host cell is an E. coli, Pantoea or Klebsiella host cell.
- 54. (Canceled)
- 55. (Previously presented): The method according to claim 52, wherein the nucleic acid molecule has the sequence of SEQ ID NO: 11 or a sequence having at least 95% sequence identity thereto.
- 56. (Canceled)
- 57. (New): The method according to claim 36, wherein said polypetide has the sequence of SEQ ID NO: 12.

- 58. (New): The method according to claim 36, wherein the bacterial host cell is an *E. coli*, *Pantoea* or *Klebsiella* host cell.
- 59. (New): The method according to claim 52, wherein said polypetide has the sequence of SEQ ID NO: 12.
- 60. (New): The method according to claim 53, wherein the bacterial host cell is a *Klebsiella* cell.
- 61. (New): The method according to claim 53, wherein the bacterial host cell is an *E. coli* cell
- 62. (New): The method according to claim 53, wherein the bacterial host cell is a Pantoea cell
- 63. (New): The method according to claim 53, wherein the bacterial host cell is deficient in endogenous 2,5 DKG permease activity.
- 64. (New): The method according to claim 53, wherein the bacterial host cell further comprises a nucleic acid molecule encoding a polypeptide having 2-keto reductase activity and at least 95% sequence identity to SEQ ID NO: 14.
- 65. (New): The method according to claim 53, wherein the bacterial host cell further comprises an isolated nucleic acid molecule having 5-keto reductase activity and at least 95% sequence identity to SEQ ID NO: 16.
- 66. (New): The method according to claim 53, wherein the bacterial host cell expresses an enzyme that catalyzed the conversion of 2,5-DKG to 2-keto-L-gulonic acid (2-KLG).

67. (New): The method according to claim 53, wherein the nucleic acid molecule encoding the protein having 2,5-DKG permease activity is operably linked to a lac promoter